

## Inactivation of Incompatibilities in Tetraploid Progenies of *Petunia axillaris*<sup>1</sup>

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During the past five years the writer and his assistants<sup>2</sup> have carried on a series of investigations to obtain direct experimental data from diploid and derived autotetraploid progenies of *Petunia axillaris* on the effect which autotetraploidy has on the heredity and expression of self- and cross-incompatibilities.

Two (6, 9) preliminary reports of these studies were made in 1941 and 1942. Since then further cultures have been studied especially in regard to the inter-group relations between the  $2n$  and the  $4n$  populations. The results obtained to date on these matters may be summarized as follows:

### INTRASPECIFIC REPRODUCTION OF DIPLOID ( $2n = 14$ ) *Petunia axillaris* (Lam.) B. S. P. (*P. nyctaginiflora* Juss.)

Of this species 23 plants grown from seed obtained from Argentina and also various further pedigreed seed progenies were remarkably uniform in character and especially in the homomorphic differentiations of sexuality in the hermaphrodite flowers. These features of fundamental specificity may be represented as **AA**. Were it not for the special factors of incompatibility this fundamental specificity would give complete intra-specific reproduction.

But fully adequate tests revealed that each individual of the entire population which was studied, including successive generations, was self-incompatible and that there were also cross-incompatibilities which operate in the simplest type (the "personate" type) of intra-specific-incompatibilities known in hermaphrodite flowering plants (4). The special incompatibility factors ("*S*" factors) exist in addition to the constitutional sexuality of specificity (**AA** +  $S_x S_y$ ). These factors are self-interacting in the physiological reactions of haploid pollen tubes and diploid styles both in self-pollination and in cross-pollination. For a plant (**AA** +  $S_x S_y$ ) there is incompatible reaction for both

<sup>1</sup> Presented in greater detail at the meeting of the Torrey Botanical Club on May 17, 1944 at The New York Botanical Garden.

<sup>2</sup> Before July 1943 Dr. Clyde Chandler was technical assistant to the writer in these studies and since that date Miss Anita Appel has had this position. Most of the routine pollinations and all of the technical laboratory studies of this research have been done by them.

classes of its pollen. Also two plants of the same genotype are cross-incompatible. When two plants possess *one* factor in common ( $\mathbf{AA} + S_1 S_2 \times \mathbf{AA} - S_1 S_3$ ) there is incompatible elimination of fertilization for the pollen which carries that factor. But when there is no common *S* factor ( $\mathbf{AA} - S_1 S_2 \times \mathbf{AA} + S_3 S_4$ ) there is no incompatible reaction and the two individuals cooperate fully in reproduction as do any two members of a species or group that possesses no incompatibility factors. In the 23 plants grown from seed from Argentina there were 12 different genotypes of from one to three individuals for which there was inter-genotypic cross-reproduction.

#### AUTO-TETRAPLOIDS OF *Petunia axillaris* AND THEIR INTRA-GROUP REPRODUCTION

Induced auto-tetraploids were readily obtained by spraying buds of young seedlings of diploid ( $2n = 14$ ) *P. axillaris* with solutions containing colchicine. From one to three lateral branches which were  $4n$  were obtained on fifteen different seedlings which were otherwise  $2n$ . These seedlings were of several genotypes in regard to *S* factors.

There was complete inhibition of the reaction of self-incompatibilities in the flowers of all  $4n$  branches. The self-pollinated flowers of  $4n$  branches produced large capsules well filled with many viable seeds while the flowers of diploid branches remained fully self-incompatible. (See figure 1.)

This inactivation of incompatibility factors was inherited in seedling offspring. Seed progenies of selfed flowers of  $4n$  branches were grown for six different plants and the total of such seedlings was 212. Another seed generation, the second for the generations of induced tetraploids, totaling 85 individuals, is now in heavy bloom. Every plant of these progenies was tetraploid. Every seedling produced large capsules and many viable seeds to normal self-pollination. Several thousand of the cross-relations between tetraploid plants (intra-series, inter-series,  $4n$  parents and  $4n$  progeny, etc.) were tested by controlled pollinations. Every one of these intra-tetraploid cross-relations resulted in capsules and many viable seeds.

Evidently in these tetraploids only microspores and macrospores which possess two genomes function in the intra-group fertilizations and seed reproduction. Thus the inactivation of self- and cross-incompatibilities which arose as a discontinuous condition in induced  $4n$  branches is hereditary on the selfed offspring, which have also been  $4n$ .

#### INTER-GROUP RELATIONS BETWEEN THE $2n$ AND THE $4n$ GROUPS OF *Petunia axillaris*

In the report of 1942 (9) it was stated that in the reactions of fertilization the tetraploid  $\times$  diploid relation for the tests made entirely failed to produce

even rudimentary capsules while the reciprocal relation of  $2n \times 4n$  resulted in large capsules and many seed. But it may now be stated that of several seed plantings involving large numbers of these seeds not one seed has germinated and also that all of these seeds thus far examined have been without embryos. There is therefore not a one-way inter-fertility or "compatibility" but an inter-group sterility barrier between  $2n$  plants and their derived  $4n$  plants that is of a status already well known for  $2n$  populations of certain species, in which there are no self- and cross-incompatibilities, and the derived  $4n$ . That this



FIGURE 1. Typical plants grown from seeds and of same age from seed planting; in 7 inch pots. At left, self-incompatible diploid plants of *Petunia axillaris*. At right,  $4n$  *P. axillaris* of the second generation after induced autotetraploidy. The two large capsules shown against the inserted card are such as are obtained to all self- and intra-cross pollinations of the  $4n$  population.



barrier extends to all  $2n$  and  $4n$  genotypes of the entire species is, of course, not determined and there is some evidence that it may not do so when distinctly different genotypes are involved.

#### THE STATUS OF SPORE ABORTIONS IN HYBRIDITY AND AUTOPOLYPOIDY

An adequate review of the several distinctly different types of sterility which operate in amphiploidy and autopoloidy is not possible at this time. It should be noted that much attention has been given to the fact that when branches or seedlings of "sterile"  $F_1$  hybrids become tetraploid the flowers of such amphiploids may be highly "fertile." But in these cases the sterility involves abortion of spores and pertains to the total potential ability of individuals to function *in any relation* in seed reproduction. This is entirely distinct from the intra-specific (or intra-group) self- and cross-incompatibilities and also from the inter-specific barriers to hybridizing fertilizations. But abortion of spores and abortion of seeds may be features either (a) of inter-specific barriers which operate after successful fertilization has produced a hybrid progeny, or (b) of the irregularities of sporogenesis in both  $F_1$  hybrids and pure autopoloids.

#### INTER-SPECIFICITY FOR $2n$ AND $4n$ POPULATIONS WHEN THERE ARE NO INTRA-INCOMPATIBILITIES

That there may be the operation of a sterility barrier between closely related  $2n$  and  $4n$  populations in various cases in which there are no self- and cross-incompatibilities has been well established. Müntzing in 1936 (3) presented an extensive survey of the data on this matter and concluded that a purely quantitative chromosome doubling often results in structural and physiological changes in specificity and that different chromosome races of a single species, including experimental autopoloids, are generally separated from each other by barriers of "incompatibility and sterility." But Müntzing uses the term "incompatibility" for the inter-relationships *between* entire species or groups of individuals and he does not mention any behavior of intra self- and cross-incompatibilities though they are known in some of the  $2n$  species which he surveys. It must be stated and perhaps emphasized that self- and cross-incompatibilities are not necessarily absent or inactive in tetraploid species. Among well established natural species they may be present or absent either in species of low chromosome number or in species that are evidently polyploid, even in the same genus.

#### DATA FOR EXPERIMENTAL AUTO-TETRAPLOIDS DERIVED FROM $2n$ PLANTS WHICH HAVE INCOMPATIBILITY FACTORS

There are somewhat meagre data on the behavior of incompatibilities in autotetraploids definitely known to have arisen from diploids in which self-

and cross-incompatibilities operate. Often the data given are confined to "Self-sterility" and are indefinite, incomplete and to some extent inaccurate in this matter. Especially is this the case for the petunias.

Lewis and Modlibowska (8) consider that certain tetraploid "systematic species" (possibly some are clones) that are "self-compatible" may have arisen from  $2n$  "self-sterile" plants of *Allium*, *Tulipa*, *Hyacinthus* and *Prunus*.

Of the more reliable data for experimental autotetraploids there is one statement (8) that self-incompatibility continues to operate in derived seedling autotetraploids of *Oenothera organensis*, and a report (10) with data that both self- and cross-incompatibilities operate in a tetraploid seed progeny derived experimentally from  $2n$  plants of *Oenothera rhombipetala* Bridgeport.

There is one case reported which corresponds, in part at least, to the relationships here presented for the diploid and the autotetraploid *Petunia axillaris*. The  $2n$  clone of the cultivated pear, Fertility, has been described as "only slightly self-fertile" (5) and as able to produce a good crop of seedless parthenocarpic fruits (7). A  $4n$  clone named "Improved Fertility" that arose as a somatic bud-sport from Fertility was found to be definitely "more self-fertile" than the  $2n$  type (5, 7), able to set a full crop of fruit and seed to its own pollen, and "fully self-compatible" (8). For the inter-relations in reproduction, the  $4n$  clone Improved Fertility  $\times$  the  $2n$  clone produced no fruits (45 flowers were pollinated) but for the  $2n$  clone  $\times$  the  $4n$  clone "a full set of fruits and seeds is obtained" (7, 8).

The intra-clonal and the inter-clonal relations in fertilization of the  $2n$  pear, Fertility, and its  $4n$  bud-sport seem to be like those of the  $2n$  and  $4n$  branches of each of the 15 original plants of *Petunia axillaris* which were treated with colchicine. But further similarities are not so evident. In *Petunia axillaris* the seeds of  $2n \times 4n$  are not, it now appears, viable; in the cultivated pears the seeds derived from  $2n \times 4n$  (see text—figure 1 and table 3 of 7) are, it seems, highly viable. The  $4n$  Improved Fertility  $\times$  five diploid clones other than Fertility was highly fertile in terms of fruit and seed production to two clones, much less so as to two other clones, and fruitless to one clone. The clones of pears correspond to individual seedlings of a species which may differ in genotypic constitution.

#### DIVERSITY IN CONSTITUTION

Much remains to be learned regarding the diversity in the behavior of self- and cross-incompatibilities in experimentally produced tetraploids. Incompatibilities both self and cross are extremely diverse in character, expression, and genetical nature (4). Uniform results can not be expected for all the diverse types especially when the simpler "personate type" is compared with the complex associate type, and when amphiploidy is involved. It is well established (4) that some of both the diploid and polyploid hybrid cultivated

racess of *Petunia* have complex polygenic and heterogenic heredity of the intra-race self- and cross-incompatibilities. Also in the constitution of both the  $2n$  and the  $4n$  varieties of hybrid origin in *Petunias* there are both qualitative and quantitative differences in the recombinations of genes, chromosomes, and genomes which determine the specificity in the diverse parental species. No doubt these may determine further inter-relations on other than a purely quantitative  $2n$  and  $4n$  relation. Tjebbes (1) reports he obtained few seed and only weak seedlings in reciprocal inter-crosses between  $2n$  "*P. nyctaginiflora*" (*P. axillaris*) and two  $4n$  cultivated races. Steere (2) made inter-pollinations between a pure race of *P. axillaris* and  $4n$  cultivated races. To some he obtained little seed of which none germinated. But one race having strongly colored flowers and blue pollen (characters of the *P. violacea*) was highly cross-fertile especially when it was the pollen parent.

#### SUMMATION FOR *Petunia axillaris*

For the cultures of *Petunia axillaris* here reported the quantitative doubling of each of the genomes in diploid plants results in two profound, different, and discontinuous changes in the reactions of reproduction.

1. The two closely related populations, one diploid and the other derived tetraploid, exhibit an inter-group sterility barrier. The  $4n \times 2n$  gives no capsules; the  $2n \times 4n$  gives capsules with seeds that are empty and not viable. The  $4n$  gains a specificity status similar to that of the  $4n$  and the  $2n$  when there are no incompatibilities and similar to that of certain well-established natural species.

2. In the  $4n$  intra-relations there is inactivation of the secondary *S* factors which operate effectively in the  $2n$ . Thus the  $4n$  population gains an intra-group reproduction that is complete for the self- and intra-cross relations between members.

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#### Literature Cited

1931. TJEBBES, KLAS. Orientierende Untersuchungen über Fertilität bei *Petunia*. Botaniska Notiser 1931:174-184.
1932. STEERE, W. C. Chromosome behavior in triploid *Petunia* hybrids. Am. Jour. Bot. 19:340-356.
1936. MÜNTZING, ARNE. The evolutionary significance of autopolyploidy. Hereditas 21:263-378.
1938. STOUT, A. B. The genetics of incompatibilities in homomorphic flowering plants. The Botanical Review 4:275-369.
1939. CRANE, M. B. and THOMAS, P. T. Genetical studies in pears I. Journal of Genetics 37:287-299.
1941. STOUT, A. B., and CHANDLER, CLYDE. Change from self-incompatibility to self-compatibility accompanying change from diploidy to tetraploidy. Science 94:118.

7. 1942. Jan. CRANE, M. B., and LEWIS, D. Genetical studies in pears III, *Journal of Genetics* **43**:31-43.
8. 1942. Jan. LEWIS, D., and MODLIBOWSKA, I. Genetical studies in pears IV. *Journal of Genetics* **43**:221-222.
9. 1942. Sept. STOUT, A. B. and CHANDLER, CLYDE. Hereditary transmission of induced tetraploidy and compatibility in fertilization. *Science* **96**:257-258.
10. 1943. HECHT, ADOLPH. Induced tetraploids of a self-sterile *Oenothera*. *Genetics* **29**: 69-74.